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One-step Indirect ELISA

User Manual

Universal™ One-step Indirect ELISA Kit

(For R&D Use only)

Product Name & Catalog Number:

Catalog # Product Name Size	Content
E-R70 Universal™ One-step Indirect ELISA Kit-R With TMB Substrate (Suitable for rabbit primary antibodies) 1 kit (1000 assays)	10XCoating Buffer (10ML), Enhancer (100ML), 100X ELISA Probe-R0 (1ML), 10X Rapid Wash Solution (100ML), 100XTMB Solution Set (2ML), User Manual (One copy).
E-M70 Universal™ One-step Indirect ELISA Kit-M With TMB Substrate (Suitable for mouse primary antibodies) 1 kit (1000 assays	10XCoating Buffer (10ML), Enhancer (100ML), 100X ELISA Probe-M0 (1ML), 10X Rapid Wash Solution (100ML), 100XTMB Solution Set (2ML), User Manual (One copy).
E-G70 Universal™ One-step Indirect ELISA Kit-G With TMB Substrate (Suitable for goat primary antibodies) 1 kit (1000 assays	10XCoating Buffer (10ML), Enhancer (100ML), 100X ELISA Probe-G0 (1ML), 10X Rapid Wash Solution (100ML), 100XTMB Solution Set (2ML), User Manual (One copy).
E-R71 Universal™ One-step Indirect ELISA Kit-R With pNPP Substrate (Suitable for rabbit primary antibodies) 1 kit (1000 assays	10XCoating Buffer (10ML), Enhancer (100ML), 100X ELISA Probe-R1 (1ML), 10X Rapid Wash Solution (100ML), pNPP Solution (100ML), User Manual (One copy).
E-M71 Universal™ One-step Indirect ELISA Kit-M With pNPP Substrate (Suitable for mouse primary antibodies) 1 kit (1000 assays	10XCoating Buffer (10ML), Enhancer (100ML), 100X ELISA Probe-M1 (1ML), 10X Rapid Wash Solution (100ML), pNPP Solution (100ML), User Manual (One copy).
E-G71 Universal™ One-step Indirect ELISA Kit-G With pNPP Substrate (Suitable for goat primary antibodies) 1 kit (1000 assays	10XCoating Buffer (10ML), Enhancer (100ML), 100X ELISA Probe-G1 (1ML), 10X Rapid Wash Solution (100ML), pNPP Solution (100ML), User Manual (One copy).

Product Description:

Universal™ One-step Indirect ELISA is the latest breakthrough technology (patent pending) for identifying and quantifying either antigen or antibody. Unlike regular (classical) ELISA, the One-step Indirect ELISA not only needs no Capture antibody and secondary antibody but also replaces blocking, primary antibody binding, washing and secondary antibody binding with a rapid one-step reaction. Therefore, using Universal™ One-step Indirect ELISA kit, you can rapidly get your ELISA results around one hour. The One-step Indirect ELISA kit is a universal kit, which is suitable for most primary antibodies without labeling, as shown on the table above. Each kit is sufficient to perform 1000 assays in 96-well plates.

Application:

- Detection and quantification of antigen.
- Identification and titration of primary antibody.
- Quantification of antigen-specific antibody.

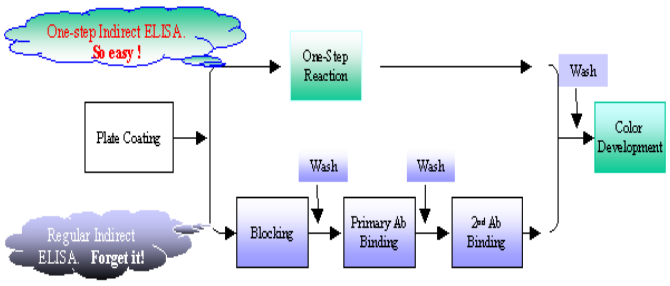
Features:

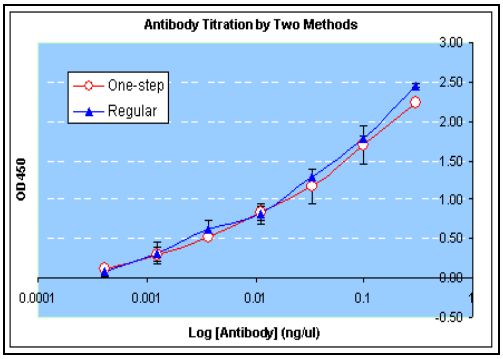
Easy: One-step reaction instead of blocking, primary antibody binding, washing and secondary antibody binding.
Rapid: Get results around one hour.
Universal: Suitable for most primary antibodies and no need of secondary antibody.
Reproducible: Highly reproducible results.

Storage:

- Enhancer: for continuous use, store in 2~8 °C for up to 3 months, or store in -20 °C for up to a year.
- 50X ELISA Probe: for continuous use, store in -20 °C for up to 6 months or store in -70 °C manual defrost freezer for up to a year. Repeated freezing and thawing is not recommended.
- Rapid Wash Solution: store in room temperature for up to one year.
  Note: Kit will be shipped in ambient temperature.

One-step Indirect ELISA v.s. Regular Indirect ELISA:





Protocol:

Materials required but not included in the kit:

- Antigen: Purified or partially purified antigen (purity = or > 30%) is recommended.
- Primary Antibody: Antigen-specific IgG Antibody. **Note:** We currently provide six types of Universal™ One-step Indirect ELISA kits. Kits labeled with -R are suitable for rabbit primary antibodies, with -M suitable for mouse primary antibodies, and with -G suitable for goat primary antibodies.
- Standard (if needed):

If test samples are antibodies, use primary antibody with a known concentration of the antigen-specific IgG.
If test samples are antigens, use purified antigen with known concentration.

- Stop solution: 1N HCl (or H2SO4) for TMB substrate or 3N HCl (or H2SO4) for pNPP substrate.
- Solid Phase: 96-well microtiter ELISA plate.

Reagent Preparation:

If test samples are antibodies:

- Coating plate: Dissolve 50-100ug antigen with 10ml coating buffer (provided in the kit) to a concentration around 5-10ug/ml. Apply 100ul of this antigen solution to each well and incubate for two hours at room temperature or overnight at 4°C, then remove the coating solution and wash wells twice with 200ul/well of 1X Rapid Wash Solution.
- 2X ELISA Probe: Dilute 200ul of the 50X ELISA Probe (red solution provided in the kit) with 5ml Enhancer (blue solution provided in the kit) and mix well. This amount is sufficient for one 96-well plate.

If test samples are antigens:

- Coating plate: Dissolve the sample antigen to be tested or standard antigen with coating buffer (provided in the kit) to make one or a series of dilutions (make 200ul each concentration at the range of 5~5000ng/ml for a duplicate tests). Apply 100ul of the diluted antigen solution to each well. After incubation for two hours at room temperature or overnight at 4°C, remove the coating solution and wash wells twice with 200ul/well of 1X Rapid Wash Solution.

  Note: Each plate must have certain wells coated with buffer alone as a negative control.

Procedure:

1. One-step Reaction:

If test samples are antibodies: Dilute the sample antibody or standard antibody with Enhancer to make one or a series of dilutions (make 100ul each concentration at the range of 0.1~1000ng/ml for a duplicate tests). **Note:** make at least 100ul Enhancer alone as a negative control for each plate. Apply 50ul each of these diluted solution (or the negative control) to each well of the antigen-coated ELISA plate, add 50ul of the above pre-diluted 2X ELISA probe to each well, and then incubate the plate for 40-60 minute at room temperature or overnight at 4°C with gentle shaking.

If test samples are antigens: Mix 3~10ug of primary antibody and 200ul of the 50X ELISA Probe with 10ml Enhancer (this amount is sufficient for one 96-well plate). Apply 100ul of this mixture to each well of the antigen-coated ELISA plate, and then incubate the plate for 40-60 minute at room temperature or overnight at 4°C with gentle shaking.

2. Wash: Wash all wells 3 times with 200ul of 1X Rapid Wash Solution (it is made by diluting 100ml of the kit-provided 10X Rapid Wash Solution with 900ml distilled water).

3. Development:

- Using TMB substrate for kits IE-R70, IE-M70 and IE-G70: Mix 100ul of 100X TMB solution A and 100ul of 100XT-buffer (B) with 10ml of distilled water, then apply 100ul of this 1XTMB mixture to each well and wait till desired color appears. Measure absorbance at 370nm (without stop) or at 450nm after stop the development by adding 100ul/well 1N HCL (or H2SO4) with a proper microplate reader.
- Using pNPP substrate for kits IE-R71, IE-M71 and IE-G71: To each well add 100ul pNPP solution and wait till desired colors appear, read the absorbance at 405nm with a proper microplate reader (optionally stop developing by adding 100ul/well 3N HCl and read the absorbance at 405nm).

Related Products:

Cat#	Product Name
WB-R51, WB-M51, WB-G51, WB-R50, WB-M50, WB-G50,	Universal™ One-step Western Blot Kits
IHC-R60, IHC-M60,IHC-G60, IHC-R61, IHC-M61, IHC-G61	Universal™ One-step IHC Kits
E-R73, E-G73, E-R74, E-G74	Universal™ One-step Sandwich ELISA Kits
E-R80, E-M80, E-G80, E-R81, E-M81, E-G81	Universal™ One-step Cell-based ELISA Kits
E-R90, E-M90, E-H90, E-G90	Universal™ One-step IgG Quantification ELISA Kits